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# GRAS Exemption Notification

# Erythritol Fatty Acid Esters for Use in Food as a Micro-Encapsulant



June 30, 2009

Stepan Company

CFR Services, Inc.
5200 Wolf Run Shoals Road
Woodbridge, VA 22192

# GRAS Exemption Notification

# Erythritol Fatty Acid Esters for Use in Food as a Micro-Encapsulant

## Table of Contents

# PROPOSED GRAS Notice

| GRN No. | Notifier | Substance | INTENDED USE | GRP # | *BASIS* | *Date* of Filing | *Closure* |
|---|---|---|---|---|---|---|---|
| | Stepan Company | Erythritol fatty acid esters | *To be used in food as an ingredient micro-encapsulant at a level not to exceed the amount required for the technical effect* | | Scientific procedures | | |

**Stepan** | Stepan Company | Northfield,Illinois 60093
Telephone 847 446 7500

JUN 3 0 2009

Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

**Attention: Paulette Gayner:**

**SUBJECT: GRAS Notification –**
**Exemption Claim for Erythritol Fatty Acid Esters**

This follows previous GRAS Notification, GRN 203, as submitted by Stepan Company, which was withdrawn from reconsideration, November 15, 2006.

A resubmission of the GRAS Notification was made March 20, 2009, following submission of this; we did receive a teleconference call from Dr. Paulette Gayner and Dr. Mike Dinovi, with respect to the need for comprehensive discussion of the published literature.

We have therefore made a revision of this GRAS exemption notice which is being sent today via FedEx with the appropriate (5) copies.

We are also providing the table of contents and the proposed abstract notice.

In accordance with FDA proposed rule of April 17, 1997 (62 FR 18938), relating to the filing of a generally recognized as safe (GRAS) notification, we hereby claim that the use of Erythritol fatty acid esters as a micro-encapsulant for ingredients added to food at levels not to exceed the amount required for the technical effect are generally recognized as safe and exempt from premarket approval requirements of the Federal Food, Drug, and Cosmetic Act. In conformity with the requirements outlined in the proposed rule, the following information is included with this exemption claim.

## GRAS EXEMPTION CLAIM

We hereby claim that the use of Erythritol fatty acid esters as a micro-encapsulant in food used at a level not to exceed the amount required for the technical effect is exempt from the premarket notification requirements of the Federal Food, Drug, and Cosmetic Act because of determination that such use of Erythritol fatty acid esters is generally recognized as safe.

(1) Identification of the notifier:

Tracy Strilich
Senior Regulatory Chemist - Product Safety and Compliance
Stepan Company
22 West Frontage Road
Northfield, IL 60093
Telephone: 847-501-2416
Faxline: 847-501-2455
E-Mail: tstrilich@stepan.com

Regulatory Representative:

William A. Olson, Ph.D.
Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Road
Woodbridge, VA 22192-5755
Telephone: 703-590-7337
Faxline: 703-580-8637
E-Mail: cfrsrv@aol.com

(2) Common or usual name of the substance:

Erythritol Fatty acid esters

(3) Applicable conditions of use:

(a) Foods in which the substance is to be used:

Erythritol Fatty acid esters are intended to be used as a micro-encapsulant of ingredients for use in foods such as baked products and confections in which the ingredient is protected against release until exposed to baking temperatures.

(b ) Purposes for which the substance is used:

Erythritol fatty acid esters are employed as micro-encapsulants for food ingredients when it is desired for release of the ingredient at elevated temperatures.

(c )Description of the population expected to consume the substance:

**Stepan**

Stepan Company

Northfield,Illinois 60093
Telephone 847 446 7500

Erythritol fatty acid esters are expected to be used in foods as consumed by the general public.

(4) Basis for GRAS determination, i.e., through experience based on common use in food (pre-1958) or through scientific procedures:

The basis of the GRAS determination is through scientific procedures.

(5) Availability of Data and Information (references)

The referenced material that is the basis for this GRAS determination is available for the Food and Drug Administration's review and copying at reasonable times at the Product Safety and Compliance Department of Stepan Company, 22 Frontage Road, Northfield, IL 60093, or will be sent to FDA upon request.

(b)(6)

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Stepan Company

## Identity of the Substance

A. Chemical Name:

Fatty Acid Diester with 1,2,3,4-butanetetrol.

B. Common Name:

Erythritol fatty acid esters

C. Trade Name:

STEPAN® EDS

D. Definition:

Erythritol fatty acid esters are prepared via two alternative routes; esterification of erythritol and fatty acids or transesterification of erythritol and methyl fatty acid esters. Erythritol fatty acid esters are a random ester of erythritol and two mole equivalents of fatty acids.

E. Description:

Pale yellow flakes

F. Specifications

| Test | Stepan Method Number | Specification |
|---|---|---|
| Acid Value | 514-O | 5 mg KOH / g Maximum |
| Saponification Value | 516-O | 158 – 190 mg KOH / g |
| Hydroxyl Value | 053-O | 120 – 200 mg KOH / g |
| Residual Alcohol (Methanol) | 149-M | 0.1% Maximum |
| Residual Methyl/Ethyl Ester | 103-G | 0.5% Maximum |
| Lead | ICP-MS | 1 mg/kg |

A summary of the analytical tests and their specification ranges are listed in Attachment No 2.

## G. Fatty Acid Profile

| Test | Stepan Method Number | Specification |
|---|---|---|
| | | |
| $C_{14}$ Myristic Acid | 499-I | 2% Maximum |
| $C_{14}$ Myristoleic Acid | 499-I | 1% Maximum |
| $C_{16}$ Palmitic Acid | 499-I | 45% Maximum |
| $C_{16}$ Palmitoleic Acid | 499-I | 1% Maximum |
| $C_{18}$ Stearic Acid | 499-I | 51% Minimum |
| $C_{18}$ Oleic Acid | 499-I | 5% Maximum |
| $C_{18}$ Linoleic Acid | 499-I | 3% Maximum |
| C18 Linolenic Acid | 499-I | 2% Maximum |
| $C_{20}$ Icosanoic Acid | 499-I | 1% Maximum |

## Chemical Description, Manufacture, Specifications, and Stability

### *Erythritol Ester Chemistry*

There are several methods for manufacturing erythritol fatty acid esters. The major processes include esterification and transesterification. The basic chemical reactions for these two methods are shown in Example 1 below:

Erythritol + Fatty acids $\rightleftharpoons$ Erythritol Esters + Water ↑

Erythritol + Fatty acid methyl esters $\overset{\text{Catalyst}}{\rightleftharpoons}$ Erythritol Esters + Methanol ↑

Example 1

Esterification is the reaction of a carboxylic acid with an alcohol. The reaction results in the formation of an ester and water. Acids, bases and heat can catalyze esterification reactions and due to the reaction equilibrium, water is distilled to isolate the desired end product, Scheme 1.

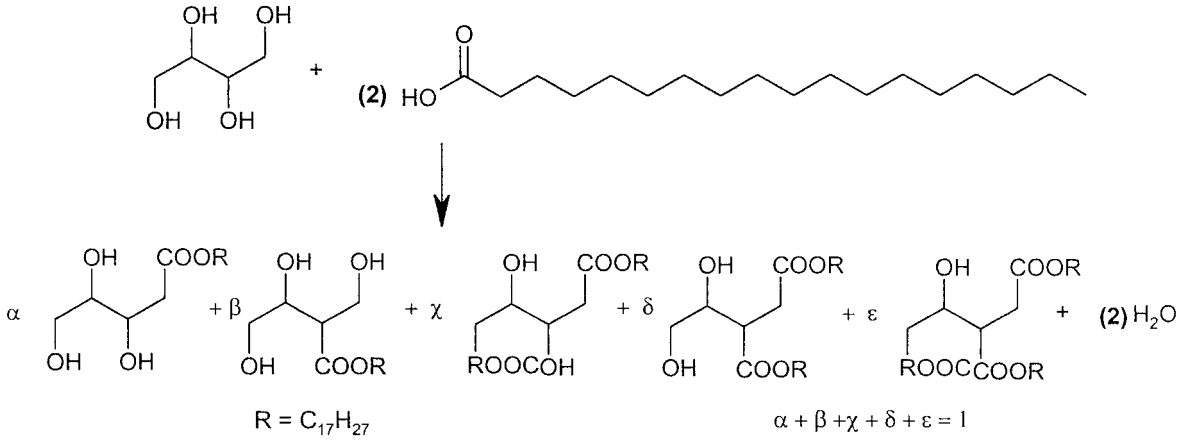

Scheme 1

A transesterification reaction is the transfer of an alkoxy group located on a carboxylic ester with one located on an alcohol. The reaction results in the formation of a new ester and a new alcohol moiety. Acids or bases can catalyze transesterification reactions and, due to the reaction equilibrium, the newly formed low boiling alcohol can be distilled to isolate the desired end product, Scheme 2.


Scheme 2

***Esterification of Erythritol and Fatty Acids***

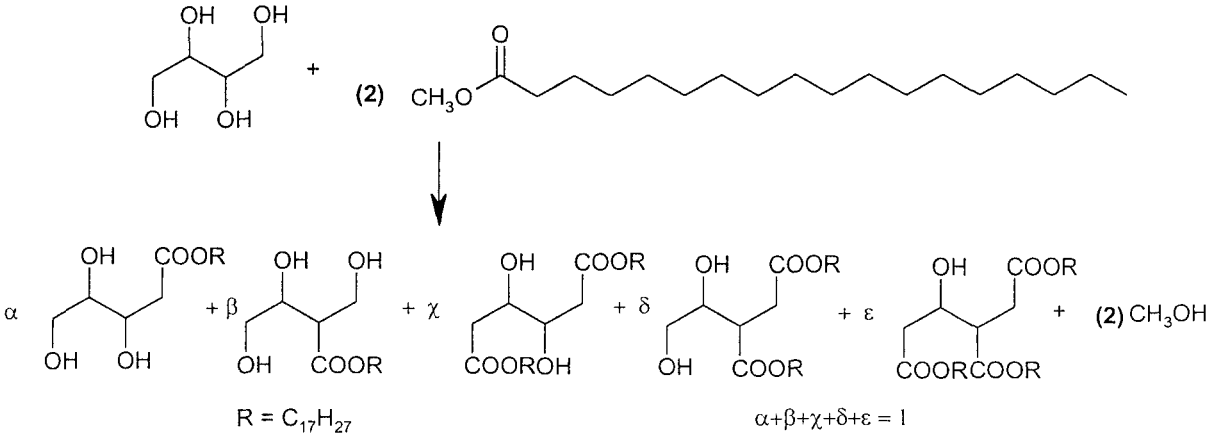
In this reaction, erythritol and fatty acid are being esterified to produce erythritol esters. The reaction is catalyzed using heat with a 2:1 mole ratio of fatty acids to erythritol. This reaction yields an indefinite ratio of erythritol monofatty acid esters, difatty acid esters, erythritol trifatty acid esters and water, Scheme 3. The water formed during the reaction is distilled out of the vessel to isolate the final product. The predominant product will be erythritol distearate due to reactivity and selectivity of the reactants. This diagram and others that follow depict a general representation of the esters produced in the end composition. Esterification in this scheme and those below happen by a random nature and the final randomized ester is a mixture of monoesters, diesters, and triesters.




Scheme 3

*Transesterification of Erythritol and Methyl Fatty acids*

Another method for producing the desired erythritol fatty acid esters is by transesterifying erythritol and a fatty acid ester, such as methyl fatty acid esters. The reaction is catalyzed using a base catalyst such as potassium carbonate, and a 2:1 mole ratio of methyl fatty acid esters to erythritol. This reaction yields an indefinite ratio of erythritol monofatty acid esters, erythritol difatty acid esters, erythritol trifatty acid esters and methanol, Scheme 4. The methanol formed during the reaction is distilled out of the vessel to isolate the final product. The predominant product will be Erythritol distearate due to reactivity and selectivity of the reactants. The residual catalysts, in the form of potassium soaps, remain in the end product mixture and are not detrimental to the finished product.



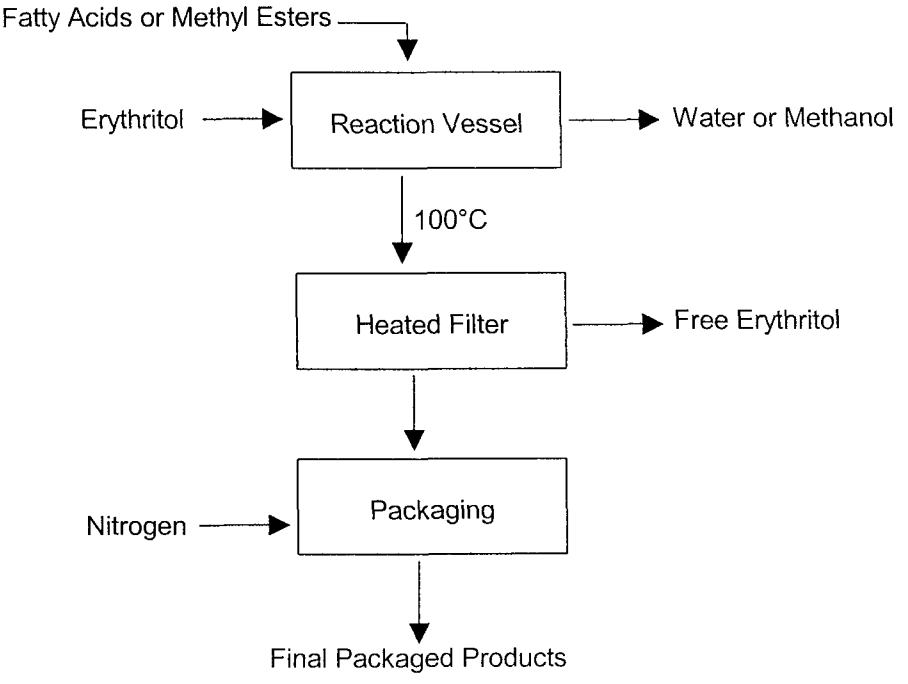

Scheme 4

***Process Description***

The process for manufacturing erythritol fatty acid esters when done by esterification takes place in three basic steps: reaction, filtering, and packaging.

A block diagram of the process is contained in Figure 1. The reaction vessel is charged with preheated fatty acid or methyl ester, to which granular erythritol is added with agitation. It is important to note that the melt point of erythritol is 122°C and will be in a suspension/slurry with the fatty acid or methyl ester until the reactor is heated to above the melt point. The reactor will be slowly heated to a final temperature of 230°C. At about 190°C, the reaction begins to generate water (or alcohol) that is removed via a total condenser. A partial condenser is also used to return any acid vapor or methyl ester to the reaction that may be carried by the reaction vapors into the reactor overheads. The batch will be held at 230°C until it is determined through acid value and hydroxyl value testing that the fatty acid or methyl ester has been essentially fully consumed. Once the reaction is complete the batch will be cooled to 100°C. A small amount of erythritol, approximately 1%, will remain unreacted and can be removed by filtration at this point; however, a small degree of erythritol remaining in the product is not detrimental. Once filtered, the erythritol ester is packaged in a flaked, prilled or drummed media for distribution and use.

**Figure 1**




The preferred route is to prepare erythritol fatty acid esters through the fatty acid condensation process as this avoids the complication of catalyst addition and subsequent removal.

***Analytical Specifications***

Lipid esters can be fully characterized by performing the four following analytical tests:

1. Acid Value
2. Saponification Value
3. Hydroxyl Value
4. Fatty acid profile by GLC

*Acid Value*

Acid value is indicative of the level of free, unreacted fatty acid remaining in the finished Erythritol fatty acid esters product. Acid value is defined as the number of milligrams of potassium hydroxide per gram of a substance required to neutralize the acids contained in that substance. The method is adapted from AOCS (American Oil Chemist Society) Method Cd 3d - 63; a copy of the Stepan method is appended. The theoretical acid value for Erythritol fatty acid esters is diminishingly small. The specification acid value for Erythritol fatty acid esters suitable for use in foods is a maximum of 5.0 mg KOH / g.

*Saponification Value*

Saponification value is indicative of the degree to which the polyol, in this case erythritol, is esterified and indicative of the equivalent weight of any lipid ester. Polyol esters can be esterified to different degrees, e.g. mono, di, tri or higher esters, depending on the number of fatty acid groups covalently bonded to the polyol. In addition, fatty acids, as obtained from natural sources, come as mixtures within a range or fraction of a range; i.e. stearic acid as obtained is primarily a mixture of saturated $C_{16}$ and $C_{18}$ fatty acids (palmitic and stearic). If one of two conditions is not met, if either the degree of esterification is too high or low or if the stearic acid does not comply with the correct fatty acid profile, then the saponification value is not likely to comply with the specification set forth in this document for an Erythritol fatty acid esters suitable for use in foods.

Saponification value is defined as the milligrams of potassium hydroxide per gram of ester required to neutralize any free fatty acid and to saponify (to make soap from) the esterified fatty acids. The method is adapted from AOCS Method Cd 3 – 25; a copy of the Stepan method is appended. The theoretical saponification value for Erythritol fatty acid esters is 175 mg KOH / g. The specification saponification value range for Erythritol fatty acid esters suitable for use in foods is 158-190 mg KOH / g.

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*Hydroxyl Value*

Hydroxyl value is the hydroxyl content of an alcohol or polyol. In the case of erythritol, there are four hydroxyls contained within the molecule. On average, erythritol fatty acid esters, a mixture of mono, di and trifatty acid esters of erythritol, has two remaining free hydroxyls and two esterified hydroxyls. Hydroxyl value is also indicative of equivalent weight, and if the conditions of degree of esterification or the fatty acid profile are not correct, then hydroxyl value is not likely to comply with the specification set forth for Erythritol fatty acid esters suitable for use in foods.

Hydroxyl value is defined as the milligrams of potassium hydroxide per gram of hydroxyl containing substance equivalent to the hydroxyl content of that substance. Hydroxyl value is performed by measuring the degree of unreacted acetate resulting from complete acetylation of a sample of the hydroxyl containing substance. The method is adapted from AOCS Method Cd 13 – 60; a copy of the Stepan method is appended. The theoretical hydroxyl value for Erythritol fatty acid esters is 154 mg KOH / g. The specification hydroxyl value range for Erythritol fatty acid esters suitable for use in foods is 120-200 mg KOH / g.

*Fatty Acid Profile*

The fatty acid profile is indicative of the distribution of fatty acids in any lipid ester. It is performed with a suitable gas chromatograph (GLC), using appropriate packed or capillary columns and appropriate detectors suitable for measuring the presence of fatty acids. In addition to identifying the presence of specific fatty acids, the relative amount of each fatty acid is determined by proportioning the peak area for each component to the total area of all components in the chromatogram. The method is adapted from AOCS Method Cd 1 – 62; a copy of the Stepan method is appended. The specification fatty acid profile for the fatty acids in Erythritol fatty acid esters suitable for use in foods is provided below in Table 1.

| Acid | Carbon Chain | Specification |
|---|---|---|
| Myristic | $C_{14}$ | 2% Maximum |
| Myristoleic | $C_{14}$ | 1% Maximum |
| Palmitic | $C_{16}$ | 45% Maximum |
| Palmitoleic | $C_{16}$ | 1% Maximum |
| Stearic | $C_{18}$ | 51% Minimum |
| Oleic | $C_{18}$ | 5% Maximum |
| Linoleic | $C_{18}$ | 3% Maximum |
| Linolenic | $C_{18}$ | 2% Maximum |
| Icosanoic | $C_{20}$ | 1% Maximum |

Table 1

*Residual Alcohol (Methanol)*

Preparing erythritol fatty acid esters by the transesterification process results in a release of alcohol rather than water, as is the case for the direct esterification process. To ensure the integrity of the final composition, it is important to verify that little or no residual alcohol remains in the product prior to quality release. This test is performed by headspace GLC of the composition. It is performed with a suitable gas chromatograph, using an appropriate capillary column and detectors suitable for measuring the presence of lower alcohols. A copy of the Stepan Method is appended. The theoretical amount of residual alcohol in erythritol fatty acid esters is diminishingly small. The specification for residual alcohol (ethanol or methanol) in erythritol fatty acid esters suitable for use in foods is a maximum of 0.1% when transesterification is the method of manufacturing.

*Residual Methyl or Ethyl Ester*

Preparing erythritol fatty acid esters by the transesterification process requires monitoring the level of unreacted alcohol ester (methanol or ethanol) to ensure the integrity of the final composition. It is important to verify that the starting ester is essentially fully consumed prior to quality release. This test is performed by GLC of the composition in a manner similar to that for obtaining the fatty acid profile; without breaking down the composition into its individual fatty acids. It is performed with a suitable gas chromatograph, using an appropriate capillary column and detectors suitable for measuring the distribution of fatty acid esters. A copy of the Stepan Method is appended. The theoretical amount of residual methyl or ethyl fatty acids in erythritol fatty acid esters is diminishingly small. The specification for residual methyl or ethyl fatty acids in erythritol fatty acid esters suitable for use in foods is a maximum of 0.5% when transesterification is the method of manufacturing.

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A summary of the analytical tests and their specification ranges are listed below in Table 2.

| Test | Stepan Method Number | Specification |
|---|---|---|
| Acid Value | 514-O | 5 mg KOH / g Maximum |
| Saponification Value | 516-O | 158 – 190 mg KOH / g |
| Hydroxyl Value | 053-O | 120 – 200 mg KOH / g |
| Fatty Acid Profile | 499-I | |
| $C_{14}$ Myristic Acid | 499-I | 2% Maximum |
| $C_{14}$ Myristoleic Acid | 499-I | 1% Maximum |
| $C_{16}$ Palmitic Acid | 499-I | 45% Maximum |
| $C_{16}$ Palmitoleic Acid | 499-I | 1% Maximum |
| $C_{18}$ Stearic Acid | 499-I | 51% Minimum |
| $C_{18}$ Oleic Acid | 499-I | 5% Maximum |
| $C_{18}$ Linoleic Acid | 499-I | 3% Maximum |
| $C_{18}$ Linolenic Acid | 499-I | 2% Maximum |
| $C_{20}$ Icosanoic Acid | 499-I | 1% Maximum |
| Residual Alcohol (Methanol) | 149-M | 0.1% Maximum |
| Residual Methyl/Ethyl Ester | 103-G | 0.5% Maximum |
| Lead | ICP-MS | 1 mg/kg |

Table 2

***Stability***

The stability of erythritol fatty acid esters was measured in two separate stability tests. The first stability test was conducted at ambient temperature. Erythritol fatty acid esters were manufactured and analyzed, held at room temperature, RT, for 11 months and reanalyzed. Results are listed below in Table 3.

| Time | 0 months | 11 months |
|---|---|---|
| StorageTemperature | @ RT | @ RT |
| Acid Value | 0.7 | 1.4 |
| Hydroxyl Value | 165.4 | 165.3 |

Table 3

The second stability test, an accelerated stability study, was conducted at an elevated temperature. Erythritol fatty acid esters were manufactured and analyzed, held at a constant temperature of 90°C for 9 months and reanalyzed. Results are listed below in Table 4.

| Time | 0 months | 9 months |
|---|---|---|
| Storage Temperature | @ 22°C | @ 90°C |
| Acid Value | 2.1 | 7.5 |
| Hydroxyl Value | 154.1 | 147.1 |

Table 4

No significant change can be observed from the above data; therefore, erythritol fatty acid esters are a stable product. The stability of erythritol fatty acid esters is equivalent to standard monoglycerides, diglycerides, and triglycerides.

**Use Information**

Materials such as triglycerides are used as encapsulating agents for food ingredients. The use of erythritol fatty acid esters as encapsulants will be only for those applications where there is requirement that the release be only at higher temperatures such as baking. The use would be similar to current use of triglyceride encapsulants, but for those uses in which the elevated melting temperature of erythritol fatty acid esters is appropriate. Carnauba wax also is used as an encapsulating agent; however, erythritol fatty acid esters offer a more consistent product with more clarity and a consistent melting point when compared to the grades of carnauba wax used for food and related applications.

Food ingredients such as flavors or leavening agents may be rendered ineffective in applications such as baking, when exposed to moisture, elevated temperatures or prolonged periods of time. In order to allow baked food to retain the flavor or leavening effect, a lipid-encapsulated component may be employed that is not released until the shell of the capsule melts. Erythritol fatty acid esters melt at a higher temperature than do glyceride esters. This enables the food formulator to use a preparation similar to esters of glycerol as encapsulants in food compositions that require processing at higher temperatures. Examples of this use include the encapsulation of flavors, leavening agents, antioxidants, or other ingredients that are added to food when activity is best expressed following exposure of temperatures in excess of 70°C. Of these uses, the primary use would be encapsulation of baking powder as a leavening agent which is estimated to be approximately 90% of the use of the erythritol fatty acid esters as encapsulants.

As outlined in Attachment 2, the chemical description section, the presently described technology relates to edible fatty acid ester compositions with erythritol. Erythritol fatty acid esters are structurally similar to glycerol esters, namely monoesters and diesters of erythritol are nearly identical to monoesters and diesters of glycerol with the exception that erythitol has an additional carbon and hydroxyl moiety. As is the case with diglycerides, erythritol partial esters are polar, and with increased polarity, there is increase in melting temperature. The preferred composition for a high melting lipid is the diesters of erythritol. As is the nature of partial fatty esters, these findings are based upon averages of the composition of commercial diglycerides which also contain molecules of monoglycerides and triglycerides, The GRAS substance is predominately dierythritides, and there are also significant inclusion of monoerythritides and trierythritides. Erythritol fatty acid esters give the best compromise of utility and melting point in that it achieves a melting point of about 80°C. The use of erythritol fatty acid esters, based upon its specialized physical characteristics, is particularly suited for use as a component of micro-encapsulation.

Erythritol fatty acid esters will replace or supplement the glyceride micro-encapsulation only when high melting point is critical and necessary to achieving the technical effect.

For this reason, erythritol fatty acid esters will not be broadly used in general food products, but instead is intended as a specialty ingredient for functional or technical foods. For example, erythritol fatty acid esters are expected to replace glycerides in the case of micro-encapsulation where high–melt temperatures are needed. Monoglycerides and diglycerides are very efficient emulsifiers and erythritol fatty acid esters will not be a direct replacement for them for food uses.

The preparation of erythritol fatty acid esters requires multiple processing steps: the isolation of fatty acids from natural food sources, the manufacture of erythritol, and the subsequent esterification of the two. Monoglycerides and diglycerides are made by a single processing step of interesterification of the naturally occurring fat with glycerin and are far less expensive to produce. On this basis, erythritol fatty acid esters are only economic in food products where the technical advantages outweigh the cost of manufacture.

***Component of encapsulation***

As the lipid component for a food microcapsule, erythritol fatty acid esters are more effective than diglycerides because of its higher melting point. When a lipid is used to encapsulate leavening agents (i.e. baking powder), the release point in the oven is governed by its melting range, and it is at this point that the active is freed from the hydrophobic matrix and can mingle with steam and water in the food product to give its full effect. Fully hardened fat (typically referred to as tristearin) has a melting point in the range of 60 – 65°C, glycerol fatty acid esters are in the range of 65 – 70°C and have therefore been a preferred fat to provide leavening encapsulation. Erythritol fatty acid esters, with an 80°C melting point, give maximum protection to the leavening agent by releasing it at the optimum time to give the greatest effect in the oven.

Foods requiring the encapsulation of baking powder include pancakes, waffles, muffins, biscuits, and popovers. Baking powder is used in these food types at up to 5 weight percent of the flour. To encapsulate the baking powder, the industry standard is 15-30% micro-encapsulant to 70-85% baking powder. Although these applications require encapsulation of baking powder, not all of these applications will require the higher-melt properties provided by erythritol fatty acid esters.. Thus, due to the higher costs of erythritol fatty acid esters, we estimate that only about 10-15 % of these baking powder encapsulated applications will benefit from erythritol fatty acid esters..

There are other examples of advantages for encapsulation with erythritol fatty acid esters. The incorporation of ω – 3 and ω - 6 fatty acids in some functional foods requires protecting the highly unsaturated fatty acid moiety from oxidative degradation during high temperature processing. An oil or fat containing these functional lipids can be commingled with erythritol fatty acid esters and prilled to make a capsule. These capsules have greater integrity than similar capsules made from hardened fat or diglycerides because of the higher melting point of the dierythritide. When used in functional foods, the capsules can withstand greater processing temperatures and higher levels of shear

than normal fat capsules. The end result is less premature release of the ω – fatty acid and, therefore, improved stability of the food to oxidative degradation.

Vitamins and other nutrients are also lipid encapsulated to protect them from oxidative degradation and extend their stability and shelf life. Further, vitamins and minerals can often contribute off-flavors to functional food products.[2] By replacing hardened fat with erythritol fatty acid esters to improve the integrity of the microcapsule, the vitamin or mineral would not be released until it is in the stomach at the time of digestion, whereas a softer and lower melting lipid would have a tendency to release the off-flavor in the cooking or eating stage of the process. All of these are advantageous uses of erythritol fatty acid esters over hardened fat or monoglycerides and diglycerides. To encapsulate the ω – fatty acids and other nutrients, the industry standard is a two-to-one ratio of micro-encapsulant to nutrient.

We estimate that erythritol fatty acid esters could take 3 – 5 % of the market for encapsulation in applications where lipids currently find utility, which is estimated to be less than 10% of the total encapsulation market. Therefore, the final market estimate for erythritol fatty acid esters is 0.3 – 0.5 % of the encapsulation market. This estimate is based on the fact that many applications do not require a higher-melt encapsulate coupled with the higher cost of erythritol fatty acid esters compared to tristearin and other high-melting lipids. Erythritol fatty acid esters require multiple processing steps (isolation of fatty acids from natural food sources, the manufacture of erythritol and the subsequent esterification). In contrast, tristearin can be made in a single step by the complete hydrogenation of soybean oil. As a result, erythritol fatty acid esters have a market price of 3-4 times the price of tristearin. This price will limit the use of erythritol fatty acid esters to those specialty applications where the technical advantages outweigh the higher price.

***Conclusion***

Erythritol fatty acid esters are structurally similar to monoglyceride and diglyceride esters and in so far as the similarity goes, they contribute to food products in ways not dissimilar to monoglycerides and diglycerides. The metabolic pathways are analogous to lipids in general but most specifically to fatty acid esters. This makes it safe for use and it should be considered GRAS for use in microencapsulation. What distinguishes erythritol fatty acid esters from the commonly used monoglycerides and diglycerides is the unique high melting point and hard crystalline nature of erythritol fatty acid esters. As outlined above, there are several specialty food applications that can take advantage of these characteristics to make the food more palatable, improve the shelf life, or enhance the nutritional characteristics. Because of the inherent additional costs to manufacture the GRAS substance, it will not be used in applications where glyceride encapsulants would be employed.

**Safety Evaluation**

Stepan Company has made the determination that erythritol fatty acid esters are generally recognized as safe.

Erythritol fatty acid esters have been determined to be generally recognized as safe through scientific procedures.

As identified in Attachment 1, this substance is produced via the esterification of erythritol with fatty acids. Erythritol is considered GRAS and was documented as GRAS under the GRAS Affirmation Petition 7G0422, with the petition being accepted for filing in the Federal Register of January 15, 1997. This petition was based upon evaluation of the safety of erythritol, the documentation being summarized and presented in the petition, GRASP 7G0422, Docket No. 97G-0063.

The comprehensive safety review of erythritol including biochemical, metabolic, toxicological, and clinical data was published in Food and Chemical Toxicology by Munro, et al. The safety data in this review included animal toxicological studies and human clinical studies. Of the animal toxicological studies, results showed that erythritol is readily absorbed, does not undergo metabolism systemically, and is excreted unchanged in urine. Also, erythritol has been shown to have no carcinogenic, mutagenic, teratogenic, or reproductive potential. The human clinical studies on erythritol showed it to be well tolerated in humans. After high dose exposures, erythritol did not present any toxicological effects. Thus, the conclusion of this safety review was that erythritol is safe for its intended use in food.

Erythritol was also evaluated for authorization of a health claim on sugar alcohols and dental caries to include the sugar alcohol erythritol. This did result in the inclusion of erythritol in the regulation relating to dietary sugar alcohols and dental caries, 21 CFR 101.80.

In addition, the GRAS Notification for erythritol, GRN 76, has been submitted by Cerestar Holding, and this GRAS Notification is effective and permits the use of erythritol as a flavor enhancer, formulation aid, humectent, nutritive sweetener, stabilizer and thickener, sequesterant, and texturizer in foods. In addition to the safety review cited above, Cerestar requested than an Expert Panel reconvene in 2000 to reassess the safety of erythritol for the expanded uses subject to their GRAS Notification. It was the conclusion of the Expert Panel that erythritol is GRAS per the expanded uses.

The fatty acid moiety in the erythritol ester is stearic acid and is consistent with the confirmed GRAS substance, stearic acid, 21 CFR 184.1090. Note that the stearic acid meets the stearic acid further defined in the Food Chemical Codex, with reference to 3rd

edition, 1981, which is updated through the 5th edition of the Food Chemical Codex, 2004, page 450.

Stearic acid, while specifically defined in the Food Chemical Codex, is organic acids contained from fats chiefly of stearic acid and palmitic acid. Thus, the fatty acids that are esterified with erythritol are considered GRAS for use in foods generally, with no restrictions excepting to be consistent with current good manufacturing practice.

With respect to in vivo cleavage of erythritol fatty acid esters, these have been studied by Mattson, et al., in a series of studies published in the Journal of Nutrition and Journal of Lipid Research. In the Journal of Nutrition study titled "Absorbability by Rats of Compounds Containing from One to Eight Ester Groups", it is shown that compounds containing one, two, or three ester groups cleaved and absorbed rapidly. Above four ester groups, cleavage and absorption decreased, and above six ester groups, cleavage and absorption stopped. Thus, with the erythritol diesters, the cleavage would be such that the products of ingestion would be erythritol and the non esterified fatty acids or fatty acid salts.

Erythritol itself is largely absorbed when excreted through the urine. The no effect level for erythritol based upon physiological effects, i.e., laxation and osmotic dieresis, is greater than 1 gram/kg body weight, or 60 grams per 60 kg person per day.

For the various uses of the microencapsulant, it is expected that the largest quantitative use of the substance is for baking powder intended for use in baked goods. Baking powder is considered a macro-additive and each one of the other types of uses would be considered a micro-additive; therefore, it is expected that the amount of use in baking powder would equal more than the total use of the encapsulant in micro-ingredients, i.e., flavors, vitamins and minerals, and polyunsaturated fatty acids.

The calculations of intake of the encapsulant used in baking powder are as follows:

The predominant use of erythritol fatty acid esters would be in the encapsulation of baking powder. The intakes of foods which contain erythritol fatty acid esters are as follows:

Pancakes & Waffles - 110 grams Total, 55% contain encapsulant, i.e., 60.5 g/person/day
Quick Breads - 56 grams Total, 8% contain encapsulant, i.e., 4.5 g/person/day
Cakes - 110 grams Total, 3% contain encapsulant, i.e., 3.3 g/person/day
Bread products - other 56 grams Total, 2 contain encapsulant, i.e., 11.2 g/person/day

Thus, the total intake of foods containing the encapsulant would be approximately 80 grams, with an estimated upper limit of 5% of each of the foods being encapsulant, this would give a total 4 grams of the encapsulated baking powder per person per day. In information previously discussed in Attachment 3, Industry Standard, baking powder is 15-30% of the encapsulant and 70-85% of the baking powder. If one used the upper estimate of 30%, this would indicate an intake of 1.2 grams/day of microencapsulant

from baking powder use. It is further estimated that only about 30% of baking powder encapsulated product will benefit from erythritol fatty acid esters, and this would give a maximum of 0.36 grams of encapsulant per 60 kg person or 6 mg/kg/body weight/day.

It is further assumed that the encapsulant from micro-ingredients would equal only approximately 10% of the use of encapsultant as used in baking powder. This would give an estimate of maximum consumer intake that would not exceed 6.6 mg/kg/body weight/day. Since 18% of the erythritol fatty acid esters are erythritol, this intake for erythritol would be 1.2 mg/kg/day. There is approximately 800 fold margin between the expected daily intake of erythritol fatty acid esters and the level which would produce any laxation or osmotic dieresis (1 gram/kg/body weight or 1000 mg/kg/body weight ÷ 1.2 mg/kg/body weight/day = 833/1).

With this slight increase in the daily intake of erythritol, there should be no effect upon the incidence of laxation or osmotic dieresis with the use of the substance as explained in this GRN Notification.

**ATTACHMENT No. 5**

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## Analytical Methods

### Stepan Company Analytical Method

| | |
|---|---|
| SM 514-0 | Acid Value and Free Fatty Acid |
| SM 516-0 | Saponification Value |
| SM 053-0 | Hydroxyl Value Determination by Acetylation |
| SM 499-1 | Carbon Chain Distribution of Esters by Gas Chromatography |
| SM 149-M | Methanol by Headspace Gas Chromatography |
| SM 103-G | Fatty Acid Methyl Esters by Gas Chromatography |
| ICP-MS | Lead by Inductively Coupled Plasma-Mass Spectrometry |

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**Stepan Company Analytical Method**
Northfield, Illinois 60093
(847) 446-7500

**SM 149 - M**

**SCOPE:**

This headspace gas chromatographic (HSGC) method determines methanol (MeOH) in various matrices, aqueous and non-aqueous alike, at 1-300 ppm levels. The results are expressed in ppm (ugrams/gram). The procedure is a standard addition approach and can be applied for MeOH determination in HCG/CG Base, methyl esters (various cuts, from C6 to C22), esterquats, esteramines intermediates and for any other matrix as long there is no chromatographic interference towards methanol or the internal standard (ISTD). Iso-butyl alcohol and n-propanol can be used as an internal standard, Remark 1.

**SUMMARY:**

A 2-gram sample of material is weighed out in triplicate; two vials are spiked at 100 (25 uL), and 200 ppm (50 uL) with 1% MeOH in IPA. An internal standard solution is added to each vial. The MeOH content is calculated using linear regression analysis for all three runs, one un-spiked and two spiked. The estimated detection and quantitation limits are 0.5 and 1.0 ppm respectively (see Remark 2)

The amount of added MeOH by "spike" is based on the expected methanol level in a sample and can be modified accordingly. The 25 and 50 uL spikes with 1% MeOH solution are suitable for MeOH content up to 300 ppm; for materials with higher MeOH content use, a more concentrated MeOH solution but do not exceed the the spike volume of 50 uL.

The methanol partition between the vapor and liquid phases in headspace procedures is matrix sensitive. In some cases, when analyzing a series of samples of the same matrix, e.g. methyl esters, a 3-point external calibration curve can be derived for one sample (one un-spiked and two spiked) and used to determine MeOH in other samples from one un-spiked run.

**SAFETY:**

METHANOL, ISOPROPANOL and iso-BUTANOL are FLAMMABLE and POISONOUS. Avoid eye and skin contact. Avoid open flames and sparks. Wear proper personal protective equipment. Work in a hood or well ventilated area.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

**APPARATUS:**

1. Gas Chromatograph, Agilent 6890 GC or equivalent equipped with an FID, a volatile interface (as an inlet) and electronic pressure control (EPC) and a computer workstation
2. Programs on computer workstation, HP GC software, version

A.03.03 or higher; Microsoft Windows, version 4.0 of higher; Microsoft Excel, version 5.0 or higher

3. Headspace analyzer, HP 7694 or HP 19395 or equivalent, with a 1 mL sample loop

4. Column, DB-WAXETR, 30 m x 0.25 mm, df = 0.25 microns

5. Flask, volumetric, 10 mL

6. Pipet, positive displacement, 25 uL, 1 mL and 5 mL (25 uL pipet must have a glass tip)
7. Analytical balance, +/- 0.0001g

8. Headspace vials, 20 mL, Teflon/butyl septa, crimp caps, open top, vial crimper and decrimper

**REAGENTS:**

1. Methanol, HPLC grade

2. Internal Standard (ISTD), iso-butyl alcohol (2-Methyl-1-propanol), purity > 99% or n-Propanol, HPLC grade

3. Isopropanol, HPLC grade
4. Citric Acid, 50% aqueous solution (only for samples containing free amines such as betaines intermediates CG or CDO Base)

**PROCEDURE:**

A. INSTRUMENT CONDITIONS for COMPUTER CONTROLLED 6890 GC (with EPC):

1. Install the capillary DB-WAXETR into the inlet and detector of the GC oven according to the column and instrument manufacturer's recommendations.

2. Program the 6890 GC using the following pages of Instrument Edit screen as follows:

Select Injection Source: Manual and enter the location of transfer line (e.g. Back).

Edit Parameters:

Columns (Carrier Flows): Install electronically an appropriate column (e.g. Back or "2")

a. Mode: Ramp Flow
Select the inlet and detector in use.

b. Inlet (Injection Port, Volatile Interface):

| | |
|---|---|
| Temperature | 220 ºC |
| Split Ratio | 30:1 |

c. Column (Carrier Flows):
Mode: Ramp Flow (Helium)

| | |
|---|---|
| Flow 1 | 1 mL/minute 11.6 psi @ 40 ºC |
| Time 1 (Hold) | 0.05 minutes |
| Ramp 1 | 5.0 mL/minute/minute |
| Flow 2 | 3 mL/minute |

| | |
|---|---|
| Time 2 (Hold) | 0.1 minutes |
| Ramp 2 | 10.0 mL/minute/minute |
| Flow 3 | 1 mL/minute |
| Time 3 (Hold) | 9 minutes |
| Ramp 3 | 10.0 mL/minute/minute |
| Flow 4 | 3 mL/minute |
| Time 4 (Hold) | 5.0 minutes |

d. Oven:

| | |
|---|---|
| Initial Temperature | 40 ºC |
| Hold Time | 6 minute |
| Ramp 1 | 10 ºC/minute |
| Temperature 2 | 70 ºC |
| Time 2 | 1.0 minutes |
| Ramp 2 | 60 ºC/minute |
| Temperature 3 | 250 ºC |
| Time 3 | 1.0 minutes |
| Run Time | 14 minutes |

e. Signal:
Select Save Data for the FID in use
Select Rate: 10 Hz

f. FID Detector:

| | |
|---|---|
| Temperature | 250 ºC |
| Air Flow | 350 mL/minute |
| Hydrogen Flow | 30 mL/minute |
| Nitrogen Flow (make-up gas) | 20 mL/minute |

g. Aux:
Select Aux Channel designated for vial pressure
Initial: 15 psi, Hold 3.0 minutes
Ramp 1: 15 psi to 5 psi, Hold 10.0 minutes

Options:
Select psi units pressure for Aux Channel

3. Program the HP 7694 headspace sampler conditions as follows:

| | |
|---|---|
| Sample oven: | 85 ºC |
| Sample valve: | 140 ºC |
| Transfer line: | 150 ºC |

Timing (sampling) in minutes for headspace autosampler:

| | |
|---|---|
| GC Cycle: | 20.0 |
| Sample Equilibration: | 10.0 |
| Vial Pressurization: | 0.20 |
| Loop Fill: | 0.20 |
| Loop Equilibration: | 0.05 |
| Sample Inject: | 1 |
| Oven Stabilization: | 1.0 |
| Agitation: | High |
| Injections per Vial: | 1 |

B. STANDARD AND SAMPLE PREPARATION:

INTERNAL STANDARD SOLUTION (ISTD solution):

1. In an appropriate container (e.g. 20 mL scintillation vial) mix well 50 uL (use 25 uL x 2 or 50 uL positive displacement micro pipet) of iso-butyl alcohol (or n-propanol) and 5 mL (positive displacement pipette) of HPLC grade isopropanol. This is the internal standard solution.

SAMPLE AND CALIBRATION STANDARD PREPARATION:

Methanol, Spiking Solution (1%, 10 ug/uL):

1. Accurately weigh 0.08 - 0.10g, +/- 0.0001g, of methanol into a 10 mL volumetric flask. Dilute to volume with IPA and mix well. This is the methanol spiking solution. It can be stored in a refrigerator up to 2 weeks.

2. Accurately weigh 2.0 +/- 0.0050 gram aliquots of the sample for methanol analysis into three separate headspace vials.

3. Prepare the "spiked vials" by adding the methanol spiking solution in 25 uL increments using a positive displacement micropipet:

| | |
|---|---|
| Level 1 | un-spiked ("as is") |
| Level 2 | 25 uL (100 ppm) |
| Level 3 | 50 uL (200 ppm) |

4. Add 25 uL of the ISTD solution to each vial using positive displacement micropipet.

5. For samples containing free amines such as HCG or CG Base, add by pipet 1mL of the 50% citric acid solution to each vial

6. Close the vial and shake vigorously (or use Vortex mixer) for 10 seconds. These are the Methanol Calibration Solutions.

C. STANDARD and SAMPLE ANALYSIS:

ChemStation/Calculation Abstract:

Cal. Table, methanol concentration: ppm (ugrams/gram)
Internal standard concentration, amount: "1"

Sample weight: not entered, all calculations are based on 2-gram sample size

Calculated Report: ISTD, results are reported in ppm (ugrams/gram

Multiplier/Dilution factor: 1

1. Program the GC as stated in PROCEDURE A, INSTRUMENT CONDITIONS, using GC manual or on-line help. Select the ISTD Report from Data Analysis. Save the procedure as a Method under the specific name.

2. Inject the following Methanol Calibration Solutions (3 vials)

| | |
|---|---|
| Level 1 | un-spiked |
| Level 2 | 25 uL |
| Level 3 | 50 uL |

3. Calculate the amount of methanol added, in ppm, to each calibration solution (Level 2 and 3) as stated in the CALCULATIONS section.

4. Using Data Analysis/Graphics features, identify the methanol and an internal standard peak (iso-butyl or n-propanol alcohol), according to the attached GC profile of Appendix 1 or 2.

5. Set up the integration events so only these two peaks are integrated, MeOH and ISTD (iso-BuOH or n-PrOH).

6. Using the Data Analysis/Cal. Table, determine the initial amount of methanol (in ppm) in a sample as follows:

Construct a 3-level calibration table for MeOH with iso-BuOH (or n-PrOH) as an internal standard. Enter methanol amounts, 0.001, for Level 1 and the calculated methanol amounts (in ppm) added to vials for Level 2 and 3 (spiked vials); for ISTD the amount is "1" Make sure the integration was done correctly for both components at each level. The initial content of methanol (in the sample) is calculated using linear regression capability of Microsoft Excel (by exporting the calibration table into a Microsoft Excel spreadsheet) or a hand calculator

7. When analysing a series of samples of the same matrix e.g. methyl esters, the calibration curve derived in Step 6, can be re-constructed as an external calibration and applied as follows:

a. Enter the initial MeOH content (determined in Step 6) for Level 1("as is" sample) and for Level 2 and 3 enter the calculated MeOH amounts for each level plus the initial amount.

b. Select Data Acquisition and Data Analysis from the method's Run Time Checklist; select the ISTD option for calculated quantitative results and Printer as a Report destination from Report menu.

c. Inject the sample solution according to Run Time Checklist of the method. The quantitative results, in ppm (ugram/gram), will be printed automatically upon completion of the run.

**CALCULATIONS:** The calculations listed below can be performed using Microsoft Excel template (see Appendix 3).

Calculate the Methanol (MeOH) concentration in the spiking solution(10 mL vol. flask):

1. MeOH in SS = WT(MeOH) x P(MeOH) x 100

Where:

MeOH in SS = MeOH Concentration in mg/mL or ug/uL in the Spiking Solution

WT(MeOH) = Weight, g of MeOH in the Spiking Solution

P(MeOH) = Purity of MeOH Reagent, Expressed as a Decimal Fraction ("1" for MeOH >99.99%)

100 = Dilution/Unit Conversion Factor

2. Calculate the amount of MeOH added to the calibration standards (added via spikes)to a 2-gram sample in ppm (ug/gram):

MeOH(Level 2) = MeOH in SS x 25 /2

MeOH(Level 3) = MeOH in SS x 50 /2

Where:

MeOH in SS = MeOH Concentration in the Spiking Solution (ug/uL)

25 and 50 = Amount of Spiking Solution Added to Each Spiked Vial in uL

2 = Sample Weight, g

3. Set-up a linear regression curve (use Excel template or hand calculator) and calculate the peak area ratio of MeOH/ISTD vs. amount ratio for all three levels. The ISTD amount is entered as "1", therefore, the curve is area ratio (y's) vs. MeOH amount (x's)

Initial Amount = Intercept/Slope

4. For Section C, Step 7: re-construct calibration curve as follows:
enter the initial MeOH amount for Level 1, for Level 2 and 3 the addeded amount (by spike) plus the initial comntent.
The curve should include the origin.

The amount (ppm) of MeOH in a sample is read from the curve using area ratio (MeOH/ISTD from the sample run).

Steps 1 through 3 can be performed automatically using "Methanol.xls" spreadsheet (see Appendix 3).

**PRECISION and ACCURACY:**

The procedure is based on a standard addition approach and the spread of response factors over the investigated range for individual samples was used to determine indirectly the accuracy and precision of the method. The RSD for the response factors derived for soybean methyl esters was found to be less than 5% at methanol levels 5 -750 ppm using 100 and 200 ppm spikes, ref. #2614-66.

The precision of multiple determinations using standard addition approach was not determined.

**REMARKS:**

1. n-Propanol and iso-butyl alcohol can be used as internal standards. n-Propanol is not suitable for esterquats that contain percent levels of isopropyl alcohol.

2. The limit of detection (LOD) and limit of quantitation (LOQ) of MeOH is somewhat matrix dependent. The LOD and LOQ are roughly estimated from the area counts of un-spiked materials; for methyl esters, they are 1 and 5 ppm respectively. None of the examined samples had methanol content less than 4 ppm.

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Lead Analysis Method

The amount of lead is determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). ICP-MS is used for the detection of trace metals. The instrument generates a plasma, which is a gas in which atoms are present in an ionized state. A droplet of a nebulized solution of the material of interest enters the ICP and evaporates. Ay solids that were dissolved in the liquid vaporize and then break down into atoms. The MS then separates and detects the ions present.

SUBMISSION END